Current Biology 23, 614–619, April 8, 2013 ©2013 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2013.02.048

Report

Nonsynaptic Plasticity Underlies a Compartmentalized Increase in Synaptic Efficacy after Classical Conditioning

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Summary

It is now well documented in both vertebrates and invertebrates that nonsynaptic as well as synaptic plasticity can be a substrate for long-term memory [1–4]. Little is known, however, about how learning-induced nonsynaptic plasticity can lead to compartmentalized presynaptic changes underlying specific memory traces while leaving other circuit functions of the neuron unaffected. Here, using behavioral, electrophysiological, and optical recording methods, we show that the previously described learning-induced depolarization of a modulatory neuron [5] of the *Lymnaea* feeding system affects its axonal terminals in a spatially segregated manner. In a side branch of the axon of the cerebral giant cells (CGCs), classical conditioning of intact animals reduced proximal-to-distal attenuation of spike-evoked calcium transients, providing a highly effective mechanism for a compartmentalized increase in synaptic efficacy. Somatic depolarization by current injection, which spreads onto the CGC's axonal side branch [5], and the blocking of A-type potassium channels with 4-aminopyridine had an effect similar to learning on the calcium transients. Both of these experimental manipulations also reduced axonal spike attenuation. These findings suggest that the voltage-dependent inactivation of an A-type potassium current links global nonsynaptic changes to compartmentalized synaptic changes.

Results

We hypothesized that learning-induced nonsynaptic plasticity in the cerebral giant cells (CGCs) [5] was associated with a mechanism that increases synaptic efficacy in its cerebral axonal branch that has a gating-in role in conditioned feeding (Figures 1A and 1B) without affecting the neuron's modulatory role in unconditioned feeding [6]. However, the nature of this mechanism remained unknown and was therefore investigated in the present study.

Previous work provided in vitro electrophysiological evidence for the presynaptic function of the CGC's axon terminals, which are thought to be involved in long-term memory [5]. Here we used optical methods to test whether these terminals were sites of presynaptic activity also in native preparations, where they are potential targets for learning-induced changes. We injected the CGC with Alexa Fluor 488 to visualize its axonal arborization, followed by bath application of the synaptic marker FM4-64 to stain active synapses in the whole ganglion. Confocal imaging revealed that FM4-64-positive red-fluorescing spots 1–4 μm in size colocalized with the green-fluorescing Alexa signal present in distal ramifications of the cerebral side branch of the previously activated CGC (Figure 1C). This finding lent experimental support to the notion that this axonal compartment has a presynaptic function in native preparations and thus further justified its targeting in our subsequent experiments.

Learning Reduces Attenuation of Spike-Triggered Axonal Calcium Transients

We directly tested the hypothesis that classical conditioning of intact animals decreases the attenuation of spike-triggered Ca^{2+} transients along the cerebral side branch of the CGC. Animals were subjected to either a paired (trained group, n = 18) or an unpaired (control group, n = 15) single-trial food-reward conditioning protocol, using amyl acetate as the conditioned stimulus (CS) and sucrose as the unconditioned stimulus (US). Combined electrophysiological and optical recording experiments started at 24 hr after training (trained group, n = 10 preparations; control group, n = 8 preparations). Separate groups of control and trained animals were subjected to behavioral tests at 24 hr after training. In these tests, the trained animals (n = 8) gave a significantly stronger feeding response to the CS compared to controls (n = 7; difference scores: 3.5 ± 0.5 versus −2.7 ± 1.2 rasps/min; unpaired Student's t test: df = 13, t = 4.3, $p < 0.0008$).

In isolated central nervous system (CNS) preparations, the calcium signal recorded in the proximal segment of the CGC main axon (indicated with dashed perimeter in Figure 2A) was not altered by classical conditioning (see Table S1 available online), and it was thus used as the basis for normalization of the signals recorded in the side branch in different preparations. The axonal calcium transients evoked by spontaneously occurring soma spikes of the CGCs (Figure S1A) showed a significant proximal-to-distal attenuation in the control group, by ~36% (Figures 2A and 2B). By contrast, the attenuation in the trained group was only ~17% and did not reach significance (Figures 2A and 2B). In the most distal region of the side branch, the calcium transient was significantly larger in the trained preparations compared to the control ones, demonstrating a learning-induced reduction of the attenuation (Figure 2B). The distal-to-proximal ratio of the transient amplitudes also was significantly higher in the trained group (0.83 ± 0.08) compared to the control group (0.58 ± 0.03; unpaired Student's t test: df = 16, t = 2.66, $p < 0.02$), further demonstrating a significant reduction of calcium transient attenuation after training.

Consistent with previous observations [5, 7], the CGC's membrane potential (MP) was significantly more depolarized in the trained group compared to the control group (Figure S2). Increased MP depolarization of the CGC was significantly positively correlated with a reduced attenuation of its axonal Ca^{2+} transients (Figure 2C; Pearson's correlation test, $R^2 = 0.56$, $p < 0.05$).

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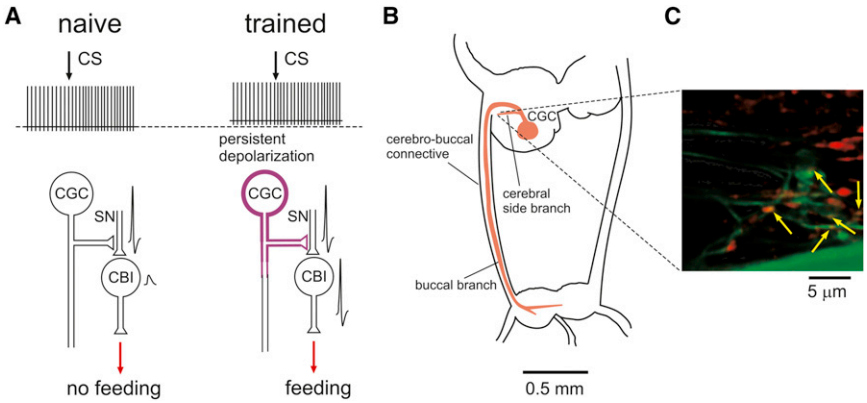

Figure 1. The Terminal of the Cerebral Side Branch of the CGC Axon Is a Presynaptic Zone Potentially Affected by Learning-Induced Nonsynaptic Plasticity

(A) The proposed mechanism of "remote-controlled" increase in synaptic efficacy after classical conditioning in *Lymnaea* (based on [5]). In naive animals, application of amyl acetate (used as the conditioned stimulus [CS] during training) leads to a small increase in the tonic firing rate of the cerebral giant cell (CGC, cartoon trace on top). Spikes evoked by the CS in putative chemosensory neurons (SN) of the lip only evoke small excitatory postsynaptic potentials in the command-like cerebral-to-buccal interneurons (CBI) of the feeding system. In conditioned animals, the CGC soma and proximal axonal segments are persistently depolarized (indicated by shifted cartoon trace on top and bold purple outline on the cartoon of the CGC), but the CS only evokes a similarly small spike frequency response, as in naive animals. However, due to increased background calcium levels in the CGC side branch, CGCs in trained animals will presynaptically facilitate the output from SNs, resulting in action-potential firing in the CBIs. The CBIs in turn will activate interneurons of the feeding CPG to produce the conditioned feeding response to the CS.

(B) The anatomical features of the axonal branching of the CGC. The main axonal projection is into the buccal ganglia (buccal branch). The cerebral side branch originates from the main axon at a distance of ~50 μm from the cell body (also see Figures 2A, 3Bi, and 4A).

(C) Example of the relationship between the signal from the red-fluorescing synaptic marker FM4-64 and the distal end of the cerebral side branch of the CGC intracellularly stained with the green-fluorescing dye Alexa Fluor 488. Arrows point at FM4-64-positive spots that colocalize with Alexa Fluor 488-stained structures and are therefore putative active synapses of the CGC axon terminal. The red spots outside the CGC axonal structure indicate the presence of synapses of neurons other than the CGC that were spontaneously active during incubation with the FM dye.

Depolarization of the CGC and 4-AP Reduce Attenuation of Axonal Calcium Transients and Spikes

To test whether depolarization of the CGC, which is sufficient to produce the network effects of behavioral classical conditioning [5], also decreases the attenuation of axonal Ca^{2+} transients, we recorded these in preparations from naive animals (n = 8), with CGCs first left at normal MP and then injected with a positive current to depolarize the soma membrane by 5 mV (this depolarization is known to spread also onto the cerebral side branch [5]). These experiments (Figure 2D) replicated the findings from the training experiments.

It was not possible to use a voltage-sensitive dye (VSD), such as JPW1114, to directly measure training-induced changes of axonal spikes, because the prolonged incubation at low temperatures necessary to get sufficient dye into the side branch (details in Supplemental Experimental Procedures) reversed learning-induced nonsynaptic plasticity. However, our hypothesis was that it is specifically the depolarization of the CGC after training that reduces both spike and calcium transient attenuation along its axon. Because the JPW1114 loading protocol did not affect the CGC's basic electrophysiological properties, we were able to use this VSD to compare axonal spikes (evoked by spontaneous somatic spikes; Figure S1B) before and after somal depolarization by current injection in the same CGCs (Figure S3). Because depolarization of the CGC by somatic current injection mimics the network effects of classical conditioning [5], it was reasonable to assume that learning-induced and artificial depolarization of the CGC affect axonal spikes similarly.

Similar to calcium transients, the optically recorded spikes (Figures S3A and S3B) showed a significantly weaker attenuation along the CGC side branch when the CGC was depolarized compared to when it was not (n = 5; Figure S3C). Importantly, the VSD-based method also revealed that depolarization did not lead to axonal spike broadening (Figure S3C) and therefore ruled this out as a mechanism underlying the increased calcium influx.

Previous work using a long-term potentiation (LTP) protocol in rat hippocampal slices indicated a role for the depolarization-induced inactivation of an A-type potassium current (I_A) in promoting the back propagation of action potentials in pyramidal neuron dendrites with a resulting increase in calcium influx [8]. We therefore hypothesized that the learning-induced reduction of attenuation of Ca^{2+} transients in the CGC axon similarly could be due to voltage-dependent I_A inactivation. To compare the possible degree of I_A inactivation in trained versus control animals, we plotted the CGC MP data obtained in the trained and control groups in the present study onto the inactivation curve of the CGC's I_A (Figure 3A), calculated from data obtained in previous voltage-clamp experiments [9]. The estimated level of inactivation was about twice as high in the trained group as in the control group (Figure 3A, insert).

To experimentally test the possible role of a reduction in I_A in the reduction of attenuation of spike-triggered Ca^{2+} transients, we applied 4-AP (0.2 mM) to naive control preparations. Attenuation was significantly smaller in 4-AP (Figure 3B), with its effect being similar to those of both training and depolarization (compare traces in Figure 3Bi to those in Figures 2A and 2D).

We used JPW1114 to verify that 4-AP affected the attenuation of axonal spike amplitude independently of affecting calcium entry (4-AP may directly stimulate high-voltage-activated Ca^{2+} channels [10]). The effects of 4-AP on axonal spikes and calcium signals were very similar (compare Figures 3C and 3Bi). The use of JPW1114 also allowed us to establish that, similar to depolarization, 4-AP significantly reduces the attenuation of axonal spikes but does not cause spike broadening (Figure S3D).

Taken together, these experiments support the notion that depolarization-driven I_A inactivation reduces the attenuation of axonal spikes and resulting calcium transients and that this therefore can serve as a mechanism underlying the effects of learning-induced depolarization of the CGC.

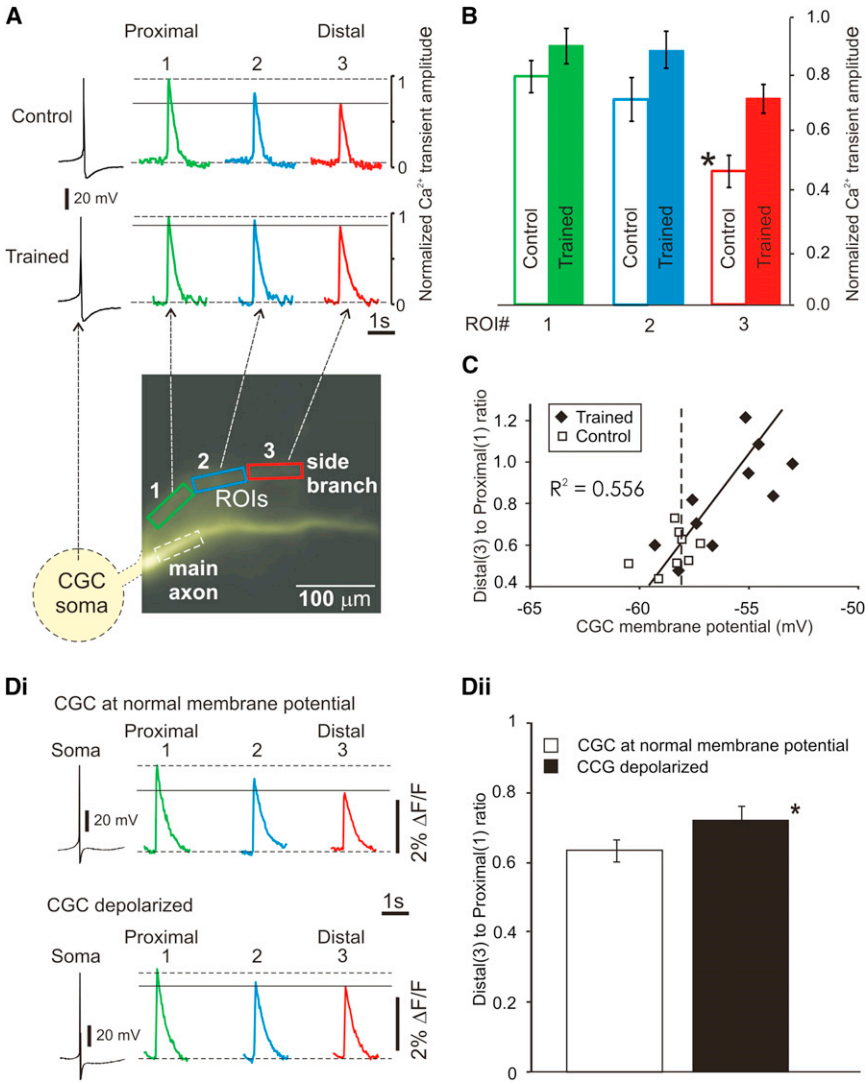

Figure 2. Effects of Classical Conditioning and Depolarization on Calcium Transients in the CGC Axonal Side Branch

(A) Examples of averaged spike-triggered calcium transients optically recorded with Oregon green from increasingly distal segments of the CGC axonal side branch (see bottom panel) and normalized to the signal in the region of the main axon outlined with the dashed perimeter. Signal attenuation between regions of interest (ROIs) 1 and 3 is smaller in the preparation from a trained animal. Dashed lines indicate no signal above baseline level and peak signal in ROI 1, respectively. Solid lines indicate the peak signal in ROI 3. The electrophysiologically recorded soma spikes that triggered the axonal calcium transients are also shown.

(B) Comparison of the attenuation of calcium transients in the axonal side branch in preparations from the control group (n = 8) and the trained group (n = 10) of animals. Mean (±SEM) values of normalized calcium signals are shown in different ROIs of the CGC axonal side branch. A two-way ANOVA detected a significant effect for both training ($F[1,48] = 12.9$, $p < 0.0008$) and the position of ROI ($F[2,48] = 10.4$, $p < 0.0002$). Bonferroni posttests revealed that the signal in ROI 3 in preparations from control animals was significantly attenuated compared to all of the other signals (*$p < 0.05$).

(C) The distal-to-proximal ratio of the normalized amplitude of calcium signals in the CGC side branch plotted as a function of the membrane potential (MP) across the whole experiment. The majority of the values from the trained group are clustered in the MP region less negative than the mean MP level of the control group (−58.4 mV, dashed line), indicating that the learning-induced parallel changes in both MP and distal-to-proximal ratio contribute to the significant overall correlation.

(Di and Dii) Depolarization of the soma membrane of the CGC by current injection significantly reduces calcium signal attenuation in the side branch. In (Dii), mean (±SEM) values obtained in eight preparations are shown. Paired Student's t test, $df = 7$, $t = 3.7$, $p < 0.007$. Note the similarity to the effect of training of intact animals.

Compartment-Specific Reduction of the Attenuation of Calcium Transients by Somatic Depolarization

To directly test the hypothesis that different axonal compartments of the CGC (Figure 4A) are affected differentially by somatic depolarization, we compared spike-evoked calcium transients in the cerebral side branch (involved in memory [5]) to those in the buccal axonal branch (involved in the modulation of the feeding circuitry [6]), at normal MP and when the same CGC soma was depolarized by ~10 mV by current injection. The amplitude of the calcium transients in the cerebral side branch was significantly larger in the depolarized CGCs compared to the same CGCs at normal MP (Figure 4B). By contrast, we found no significant difference in calcium transients evoked by single action potentials at normal versus depolarized MP in the main axonal branch at the site of its entrance into the buccal ganglia (Figure 4C). These findings directly demonstrated that depolarization selectively reduces the attenuation of spike-evoked calcium transients in the cerebral side branch of the CGC axon compared to its buccal branch. The axonal calcium transients were blocked by $CdCl_2$ (Figure S4), identifying the high-voltage-activated calcium current of the CGC [9] as the source of spike-triggered calcium influx.

Discussion

Here we have shown that the attenuation of spike-triggered calcium transients along a specific branch of the axon of a molluscan modulatory interneuron is significantly reduced by learning. This effect was positively correlated with the learning-induced depolarization of this well-identified neuron, the CGC, with a known role in associative memory in *Lymnaea* [5]. Importantly, depolarization of the CGC by current injection also reduced the attenuation of calcium transients (and underlying axonal spikes) in the cerebral side branch of the axon. However, the same level of depolarization failed to change the amplitude of spike-evoked calcium transients in the buccal branch. Thus, the cerebral side branch appears to be selectively affected by nonsynaptic plasticity. It arborizes in an area of extensive branching of neurites of cerebral-buccal interneurons [11] at the entry point of the lip nerves to the cerebral ganglia, thus providing the anatomical context for the

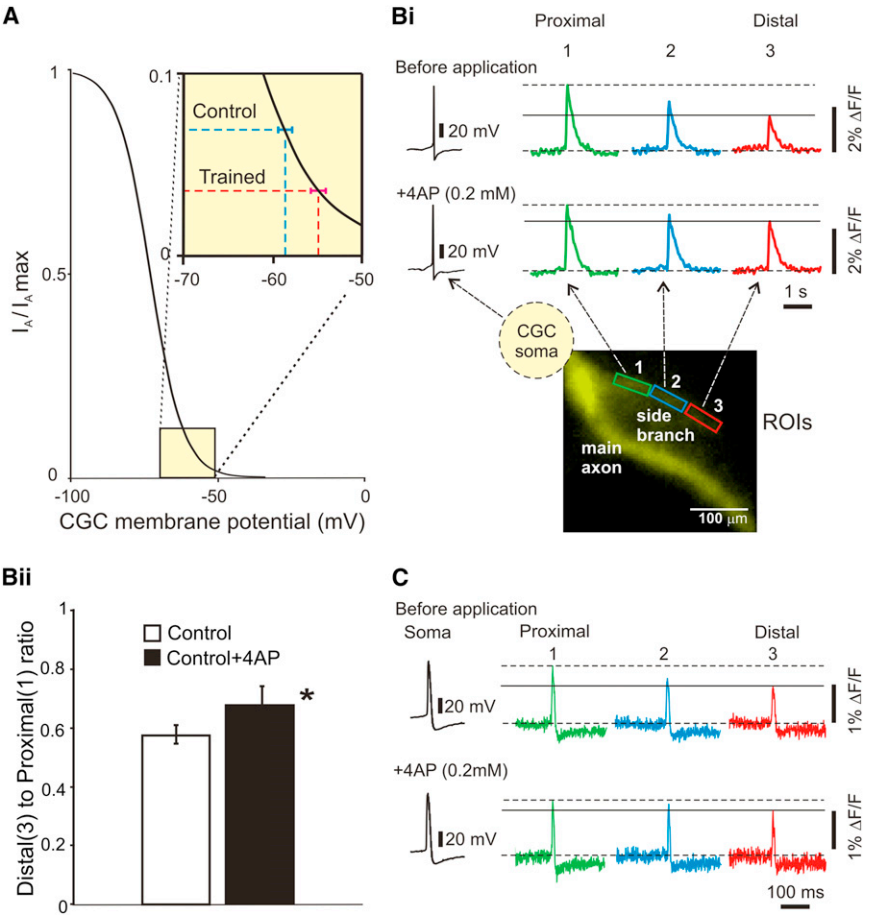

Figure 3. Suppression of a Potassium A Current Reduces the Attenuation of Calcium Transients and Spikes in the Cerebral Side Branch of the CGC

(A) The inactivation curve of the potassium A current (I_A) of the CGC (based on voltage-clamp data from [9]). Inset: CGC MP values (mean ± SEM) from preparations from control (blue lines) and trained animals (red lines) plotted on the relevant section of the inactivation curve.

(Bi) Comparison of the ratios of the calcium signal amplitudes measured at the most proximal (1) and most distal (3) regions of interest (ROIs; see bottom panel) in a preparation from a naive control animal before and after 4-AP treatment. Dashed lines indicate baseline levels and the peak of the calcium transient in ROI 1, respectively. Solid line indicates the peak of the calcium transient in ROI 3. The electrophysiologically recorded soma spikes that triggered the axonal calcium transients (recorded with Oregon green) are also shown.

(Bii) The ratio (mean ± SEM values from eight preparations) is significantly higher after the application of 4-AP (paired t test, df = 7, $t = 2.36$, $p < 0.05$).

(C) Attenuation of spikes in the cerebral side branch of the CGC and its reduction by 4-AP recorded with the voltage-sensitive dye JPW1114. Dashed lines indicate baseline levels and the peak of the axonal spikes in ROI 1, respectively. Solid line indicates the peak of the axonal spikes in ROI 3.

depolarized CGC's known ability to facilitate amyl acetate-activated lip chemosensory inputs to these interneurons [5].

In the *Aplysia* feeding network, transmission between sensory neurons and motoneurons is affected by spike attenuation, which can be reduced by central depolarization of the presynaptic neuron [12]. Similar to the *Lymnaea* CGC [5], central depolarization of the *Aplysia* B21 neuron induces graded changes in the baseline intracellular calcium concentration of its axon terminals [13]. However, whereas in *Aplysia* the role of phasic central depolarization has been investigated in the context of unconditioned feeding, in *Lymnaea* we focused on the role of persistent central depolarization in associative food-reward learning [5, 7].

Similar to our finding that axonal spike attenuation is reduced by 4-AP, a selective blocker of the CGC's I_A [9], axonal spike propagation failure in hippocampal neuronal cultures can be inhibited by applying 4-AP to the presynaptic neurons [14]. Although the mechanisms we describe here are also similar to the potassium and calcium current-based mechanisms of intrinsic dendritic plasticity in mammalian neurons [8, 15, 16], our study represents a major advance over previous work. Unlike the in vitro LTP protocols used in mammalian studies, we for the first time employed behavioral training with subsequent imaging and electrophysiological experiments in whole CNS preparations to investigate the link between central depolarization and compartmentalized synaptic changes. Moreover, the kind of LTP experiments used by Frick et al. [8] and others (e.g., [15, 16]) usually do not last longer than ~1 hr and thus deal with short- to medium-term plasticity. Our experiments, however, were performed at 24 hr after training, investigating mechanisms underlying long-term memory [5]. Another important difference is that the LTP-induced modification of I_A in dendrites of mammalian neurons is thought to be mediated by intracellular signaling cascades [8], whereas in the CGC the learning-induced persistent depolarization spreading from the soma onto the axonal compartment [5] seems to be sufficient to inactivate I_A. However, it is possible that learning also induces changes in axonal ion channels that contribute to axonal depolarization. Further work will be required to decide whether the model should be broadened to include learning-induced axonal as well as somatic nonsynaptic plasticity.

However, the most fundamental difference between previous studies investigating postsynaptic plasticity in dendrites and our study investigating the link between somal plasticity and enhanced presynaptic output in axons lies in the different nature of the computational problems that neurons have to solve when selectively regulating their inputs, as opposed to selectively regulating their outputs. Selective intrinsic dendritic plasticity results in only specific inputs being able to gain access to the spike-generator mechanisms of the neuron and plays no further role in defining the output from the cell. By contrast, synaptic output from a centrally depolarized neuron needs to be selectively regulated via some mechanism that differentially affects the propagation of spikes in different axonal branches of the neuron, independently of the input that triggered the spike activity in the first place. Our present study is the first to describe such a mechanism for this latter type of

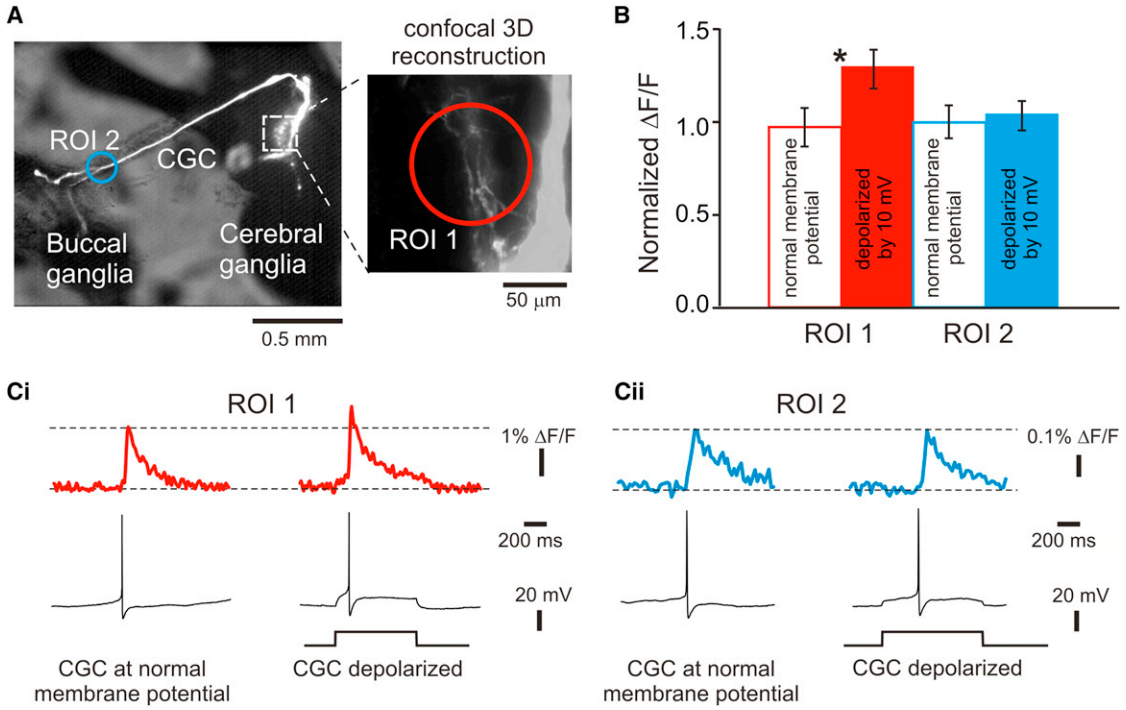

Figure 4. Comparison of the Spike-Evoked Calcium Transients in the Cerebral Side Branch and the Main Axon of the CGC at Normal versus Depolarized Membrane Potential Levels

(A) A photomicrograph of a CGC's cell body and axonal branching visualized in a native preparation with a mixture of Alexa red and Oregon green, with sites of calcium imaging indicated. Region of interest 1 (ROI 1 in insert) demarcates the site of optical recording from the side branch (cerebral projection), and ROI 2 demarcates the site of optical recording from the main axonal branch (buccal projection). On the right, the detailed 3D reconstruction of confocal microscope images of the side branch projected onto the ground plane is shown.

(B) Normalized amplitudes (mean ± SEM) of calcium transients recorded from ROI 1 and ROI 2 at normal somal MP and when the MP was depolarized by 10 mV. In each ROI, individual ΔF/F data were normalized to the mean of the data obtained in the same ROI at normal MP. Asterisk indicates a statistically significant difference between the normalized signal in ROI 1 when the CGC was depolarized versus each of the other values (ANOVA: $F[3,23] = 3.8$, $p < 0.03$; Tukey's test: $p < 0.05$ for each pairwise comparison).

(Ci) Example of an experiment with the calcium traces obtained from ROI 1. Bottom traces: action potentials in the CGC soma at normal MP (left) and with the soma depolarized by 10 mV (right). Top traces: calcium transients recorded with Oregon green BAPTA-1 in ROI 1, corresponding to the spikes in the bottom traces. Dashed lines indicate baseline levels and the peak of the calcium transient at recorded potential, respectively.

(Cii) Example of an experiment with the calcium traces obtained from ROI 2. Note that the much smaller amplitude of the calcium transients recorded in ROI 2 versus ROI 1 is largely due to the concentration of somally injected Oregon green being smaller in the more distal buccal compartment of the CGC axon compared to the more proximal cerebral compartment.

"remote-controlled" presynaptic regulatory process in native preparations from classically conditioned animals.

Recent in vitro experiments using VSD-based imaging have shown that a 4-AP-sensitive Kv1 potassium channel underlies distance-dependent changes in spike shape in cortical axon collaterals in response to somatic MP depolarization [17]. We demonstrated that the propagation of spikes in axonal branches of a molluscan interneuron is subject to attenuation that can be reduced by both somal depolarization and 4-AP. Thus, both molluscan and mammalian neurons seem to employ similar potassium-conductance-based mechanisms to link central depolarization to compartmentalized presynaptic effects, and these mechanisms also seem to be shared by mechanisms shaping compartmentalized dendritic plasticity in mammalian neurons.

Presynaptic depolarization can potentiate the synaptic output in both invertebrate and vertebrate synapses through an elevation of background calcium levels [5, 13, 18]. There are now also a number of examples, including the present study together with [5], showing that somatic depolarization increases the probability of neurotransmitter release by increasing both baseline calcium levels and spike-triggered calcium influx in proximal presynaptic terminals [19, 20]. However, our current study is the first to link this mechanism operating in an identified neuron to a specific example of behavioral learning.

Experimental Procedures

Experimental Animals and Single-Trial Food-Reward Classical Conditioning Protocol

The breeding and maintenance of the pond snail *Lymnaea stagnalis* and the details of the single-trial food-reward classical conditioning protocol used in this study have been described in a number of previous papers [5, 7, 21, 22]. We used blind procedures in each stage of the experiments, from behavioral testing through electrophysiological and optical recording to data analysis.

Electrophysiology

The current-clamp-based electrophysiological methods used in this study on the CGC have been described in a number of previous papers [5, 7, 9]. All electrophysiological experiments were performed in conjunction with optical imaging of axonal spikes or resulting calcium transients.

Calcium Imaging

We used the membrane-impermeable calcium-sensitive probe Oregon green 488 BAPTA-1 (Invitrogen) to detect fast axonal calcium transients.

For more technical details of the calcium imaging method, see Supplemental Experimental Procedures.

Optical Recording of CGC Activity with a Voltage-Sensitive Dye

The voltage-sensitive dye JPW1114 (D-6923, Invitrogen) was used to record spikes in the CGC axonal branches. For more technical details of the VSD imaging method, see Supplemental Experimental Procedures.

FM4-64 Experiments and Confocal Microscopy

The red-fluorescing styril dye FM4-64 was used to label putative spike-activated synapses in the CGC axon terminals that were visualized by intracellularly injected green-fluorescing Alexa Fluor 488. For more technical details of the combined Alexa and FM dye-based confocal imaging method, see Supplemental Experimental Procedures.

Statistical Analysis

All data sets passed the Kolmogorov-Smirnoff normality test, and therefore parametric tests were used (two-way ANOVA with Bonferroni posttest for multiple comparisons and Student's t tests for pairwise comparisons, GraphPad Prism). Differences were considered significant at $p < 0.05$.

Supplemental Information

Supplemental Information includes four figures, one table, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.02.048.

Acknowledgments

G.K. was supported by the BBSRC and MRC (UK). E.S.N. and P.M.B. were supported by RFBR, MCB, and IP grants (Russian Federation). We would like to thank T. Korshunova (Institute of Higher Nervous Activity and Neurophysiology) for assistance with the classical conditioning experiments and K. Staras (Sussex Neuroscience) for useful advice regarding the FM dye imaging experiments.

Received: October 31, 2012
Revised: January 31, 2013
Accepted: February 25, 2013
Published: March 28, 2013

References

1. Debanne, D., Daoudal, G., Sourdet, V., and Russier, M. (2003). Brain plasticity and ion channels. J. Physiol. Paris *97*, 403–414.
2. Magee, J.C., and Johnston, D. (2005). Plasticity of dendritic function. Curr. Opin. Neurobiol. *15*, 334–342.
3. Benjamin, P.R., Kemenes, G., and Kemenes, I. (2008). Non-synaptic neuronal mechanisms of learning and memory in gastropod molluscs. Front. Biosci. *13*, 4051–4057.
4. Mozzachiodi, R., and Byrne, J.H. (2010). More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. Trends Neurosci. *33*, 17–26.
5. Kemenes, I., Straub, V.A., Nikitin, E.S., Staras, K., O'Shea, M., Kemenes, G., and Benjamin, P.R. (2006). Role of delayed nonsynaptic neuronal plasticity in long-term associative memory. Curr. Biol. *16*, 1269–1279.
6. Yeoman, M.S., Brierley, M.J., and Benjamin, P.R. (1996). Central pattern generator interneurons are targets for the modulatory serotonergic cerebral giant cells in the feeding system of *Lymnaea*. J. Neurophysiol. *75*, 11–25.
7. Nikitin, E.S., Vavoulis, D.V., Kemenes, I., Marra, V., Pirger, Z., Michel, M., Feng, J., O'Shea, M., Benjamin, P.R., and Kemenes, G. (2008). Persistent sodium current is a nonsynaptic substrate for long-term associative memory. Curr. Biol. *18*, 1221–1226.
8. Frick, A., Magee, J., and Johnston, D. (2004). LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. Nat. Neurosci. *7*, 126–135.
9. Staras, K., Győri, J., and Kemenes, G. (2002). Voltage-gated ionic currents in an identified modulatory cell type controlling molluscan feeding. Eur. J. Neurosci. *15*, 109–119.
10. Wu, Z.Z., Li, D.P., Chen, S.R., and Pan, H.L. (2009). Aminopyridines potentiate synaptic and neuromuscular transmission by targeting the voltage-activated calcium channel beta subunit. J. Biol. Chem. *284*, 36453–36461.
11. McCrohan, C.R., and Kyriakides, M.A. (1989). Cerebral interneurons controlling feeding motor output in the snail *Lymnaea stagnalis*. J. Exp. Biol. *147*, 361–374.
12. Evans, C.G., Jing, J., Rosen, S.C., and Cropper, E.C. (2003). Regulation of spike initiation and propagation in an *Aplysia* sensory neuron: gating-in via central depolarization. J. Neurosci. *23*, 2920–2931.
13. Ludwar, B.Ch., Evans, C.G., Jing, J., and Cropper, E.C. (2009). Two distinct mechanisms mediate potentiating effects of depolarization on synaptic transmission. J. Neurophysiol. *102*, 1976–1983.
14. Debanne, D., Guérineau, N.C., Gähwiler, B.H., and Thompson, S.M. (1997). Action-potential propagation gated by an axonal I(A)-like K+ conductance in hippocampus. Nature *389*, 286–289.
15. Kampa, B.M., and Stuart, G.J. (2006). Calcium spikes in basal dendrites of layer 5 pyramidal neurons during action potential bursts. J. Neurosci. *26*, 7424–7432.
16. Losonczy, A., Makara, J.K., and Magee, J.C. (2008). Compartmentalized dendritic plasticity and input feature storage in neurons. Nature *452*, 436–441.
17. Foust, A.J., Yu, Y., Popovic, M., Zecevic, D., and McCormick, D.A. (2011). Somatic membrane potential and Kv1 channels control spike repolarization in cortical axon collaterals and presynaptic boutons. J. Neurosci. *31*, 15490–15498.
18. Awatramani, G.B., Price, G.D., and Trussell, L.O. (2005). Modulation of transmitter release by presynaptic resting potential and background calcium levels. Neuron *48*, 109–121.
19. Christie, J.M., Chiu, D.N., and Jahr, C.E. (2011). Ca(2+)-dependent enhancement of release by subthreshold somatic depolarization. Nat. Neurosci. *14*, 62–68.
20. Yu, Y., Maureira, C., Liu, X., and McCormick, D. (2010). P/Q and N channels control baseline and spike-triggered calcium levels in neocortical axons and synaptic boutons. J. Neurosci. *30*, 11858–11869.
21. Alexander, J.J., Jr., Audesirk, T.E., and Audesirk, G.J. (1984). One-trial reward learning in the snail *Lymnea stagnalis*. J. Neurobiol. *15*, 67–72.
22. Kemenes, I., Kemenes, G., Andrew, R.J., Benjamin, P.R., and O'Shea, M. (2002). Critical time-window for NO-cGMP-dependent long-term memory formation after one-trial appetitive conditioning. J. Neurosci. *22*, 1414–1425.